American Journal of Medical Genetics 65:40–43 (1996)

Mutation Analysis in Duchenne and Becker Muscular Dystrophy Patients From Bulgaria Shows a Peculiar Distribution of Breakpoints by Intron

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For the first time in Bulgaria, a deletion/duplication screening was performed on a group of 84 unrelated Duchenne/Becker muscular dystrophy patients, and the breakpoint distribution in the dystrophin gene was analyzed. Intragenic deletions were detected in 67.8% of patients, and intragenic duplications in 2.4%. A peculiar distribution of deletion breakpoints was found. Only 13.2% of the deletion breakpoints fell in the "classical" hot spot in intron 44, whereas the majority (>54%) were located within the segment encompassing introns 45–51, which includes intron 50, the richest in breakpoints (16%) in the Bulgarian sample. Comparison with data from Greece and Turkey points at the probable existence of a deletion hot spot within intron 50, which might be a characteristic of populations of the Balkan region. © 1996 Wiley-Liss, Inc.

KEY WORDS: Duchenne muscular dystrophy, Becker muscular dystrophy, dystrophin deletions, dystrophin duplications, breakpoints

INTRODUCTION

Most Duchenne (DMD) and Becker muscular dystrophy (BMD) cases are due to partial deletions of the dystrophin gene [Koenig et al., 1989; den Dunnen et al., 1989]. It is well-known that introns 7 and 44 are preferentially involved as origins of breakpoints [Wapenaar et al., 1988; Gillard et al., 1989]. However, when considering the distribution of breakpoints by intron, differences were detected among different populations of Western Europe [Danieli et al., 1993]. Moreover, a significantly lower proportion of dystrophin gene deletions was recently reported for the Israeli population, compared to Europe and North America [Shomrat et al., 1994], supporting the hypothesis that a differential deletion pattern for such a gene might exist in different human populations.

We describe here the results obtained from the study of a cohort of Bulgarian patients affected with DMD or BMD. This is the first report on distribution of intragenic deletions and duplications in this population.

PATIENTS AND METHODS

During a 5-year period, 84 unrelated Bulgarian families with DMD/BMD were referred to the Laboratory of Molecular Pathology, Sofia, for detection of molecular defects in affected males and for assessment of carrier status in female relatives.

Clinical diagnosis was performed in the Faculty Hospital of Child Neurology, Sofia, in the Faculty Pediatric Hospital, Sofia, and in some regional neurological clinics in different parts of Bulgaria. Classical clinical criteria were adopted for assignment to the DMD or to the BMD group: age-of-onset, age of becoming wheelchair-bound, serum creatine kinase (CK) levels, electromyography (EMG), and muscle biopsy. Seventy-one patients were diagnosed with DMD, and 13 with BMD. All subjects were from Bulgaria and were born to Bulgarian families.

DNA was extracted from white blood cells, according to a standard salting-out procedure.

Deletion screening was performed by multiplex polymerase chain reaction (PCR) amplification of 18 exons, corresponding to different regions of the dystrophin gene [Chamberlain et al., 1988; Beggs et al., 1990]. The amplified exons were: 3, 4, 6, 8, 12, 13, 17, 19, 43, 44, 45, 47, 48, 49, 50, 51, 52, and 60. Some of the deletion breakpoints were precisely detected by additional primers identifying exons: 1, 2, 5, 7, 11, 16, 21, 25, 29, 32, 34, 41, 42, 46, 53, 54, 55, 56, 57, and 59. After 8% polyacrylamide gel electrophoresis, the gels were

Received for publication June 20, 1995; revision received September 29, 1995.

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silver-stained for visualization of the amplicons, according to Saad et al. [1993].

Duplication detection was also performed by multiplex PCR amplification followed by silver-staining, according to Galvagni et al. [1994], but some modifications were introduced: 80–100 ng of template DNA were used, and 18 cycles of amplification were chosen as a limiting condition for purposes of quantitation [Bronzova et al., 1994]. Under these conditions, differences in intensity were directly visible, without the aid of a densitometer.

RESULTS

The study involved 84 unrelated patients: 71 affected with DMD, and 13 affected with BMD. Intragenic deletions were detected in 57 subjects (67.8%), and intragenic duplications in 2 (2.4%). In total, 106 deletion breakpoints were precisely identified and assigned to specific introns. Eight breakpoints (7%) could not be defined with the primers available for the study.

According to previous studies on the same subject, the entire gene was subdivided into three sections: from the 5′ end to exon 20, from exon 21 to exon 40, and from exon 40 to the 3′ end. Sixteen percent of deletion breakpoints fell in the first section and 84% in the mid-distal part of the gene, whereas no breakpoints were detected in the section encompassing introns 20–40. Two deletions were found to start in the promoter region: one upstream −217, and the other downstream +94. The deletion starting upstream −217 ended within intron 44, while the second ended within intron 1. No deletion was found in the segment downstream exon 55.

Localization of the 57 intragenic deletions and two duplications detected in the Bulgarian DMD/BMD patients is shown in Figure 1, and the distribution of deletion breakpoints by intron is shown in Figure 2.

Intron 50 appeared to be the richest in breakpoints (16.0% of total), followed by intron 44 (13.2% of total). We focused our attention on two regions, intron 44 and the segment between introns 45–51, the sizes of which are almost completely defined [Nobile et al., 1995] and comparable. The breakpoint distribution along these segments appeared to be very different. The majority (54.7%) of deletion breakpoints were located within the segment encompassing introns 45–51, which includes intron 50, while only 13.2% of them fell in the "classical" hot spot corresponding to intron 44.

Of the two intragenic duplications detected in the Bulgarian sample, one included exons 2–32 (or possibly 33), and the second involved only exon 50.

DISCUSSION

The frequency of intragenic deletions among the Bulgarian patients affected with DMD or BMD did not differ significantly from the values reported in the literature for European populations [Claustres et al., 1991; Vitiello et al., 1992; Nicholson et al., 1993]. On the contrary, the frequency of duplications appeared lower than expected [Galvagni et al., 1994]. Only two intragenic duplications were observed, while six were expected on the basis of the data reported in the literature. However, the limited size of the sample makes it difficult to assess the real significance of this difference.

On the other hand, the distribution of breakpoints by intron was quite different from what has been reported for the average European population [Danieli et al., 1993]. In the Bulgarian sample, there was a peculiar concentration of breakpoints in intron 50, and a relative paucity of breakpoints in intron 44.

The comparison with breakpoint distributions obtained from the study performed in Greece [Florentin et al., 1994] shows that in this sample there is also a high incidence of deletion breakpoints in intron 50 (Fig. 2b), whereas the sample of DMD/BMD cases from Turkey [Gökgöz et al., 1993] showed a noticeable incidence of

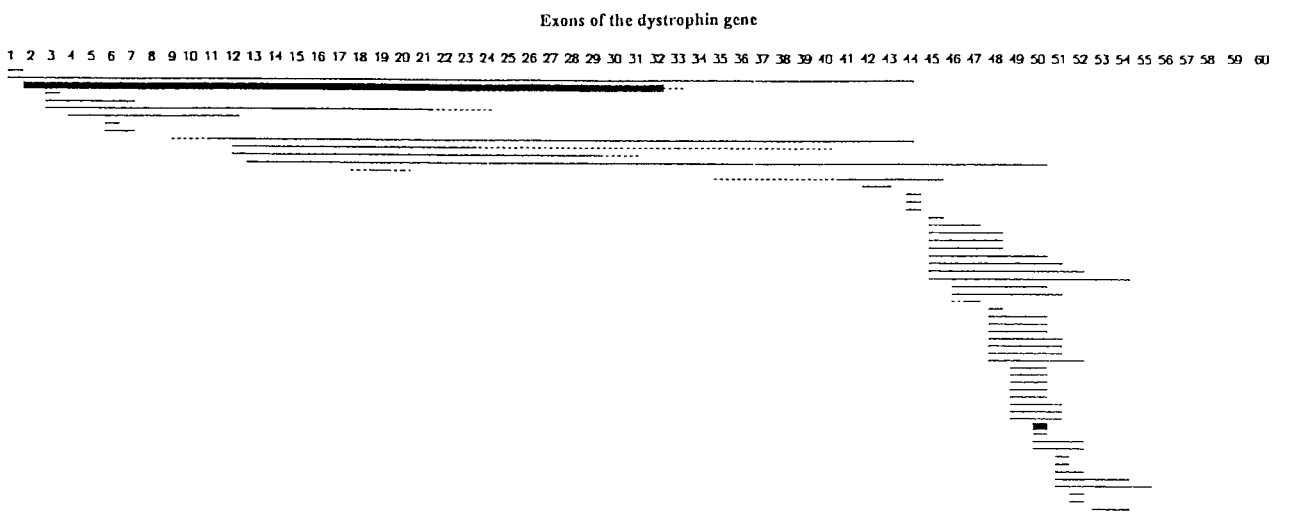

Fig. 1. Size and position of 57 deletions and two duplications detected in 84 unrelated Bulgarian patients affected with DMD/BMD. Lines, extension of deletions; bars, extension of duplications; dotted lines, imprecisely defined borders.

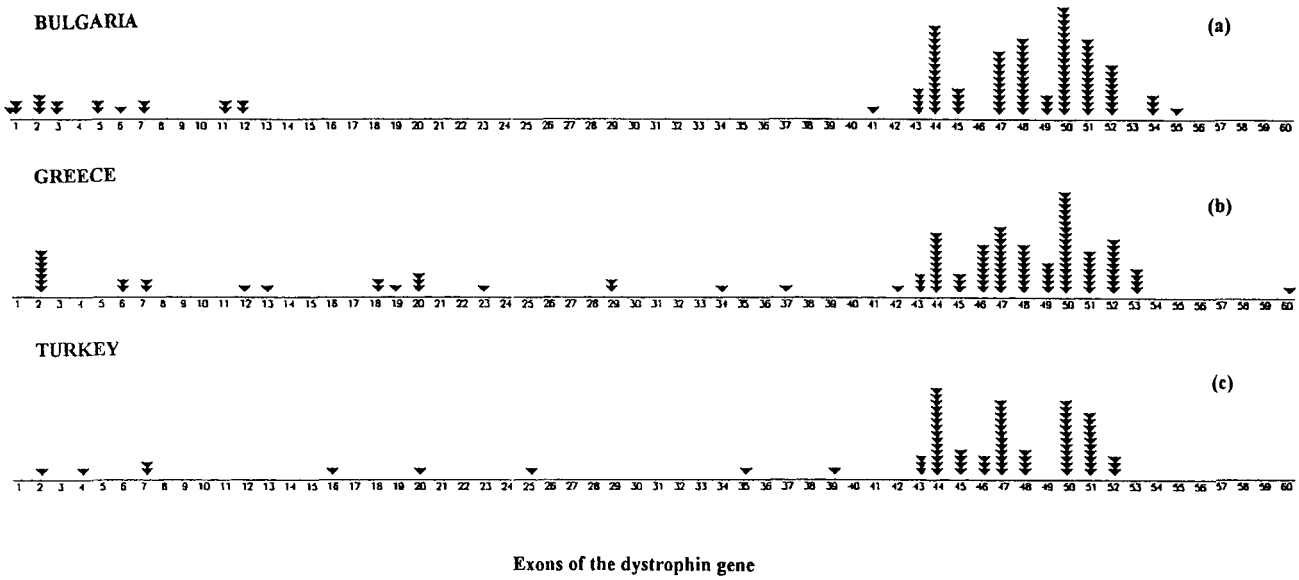

Fig. 2. Deletion breakpoints distribution by intron along the dystrophin gene in Bulgarian (**a**), Greek (**b**), and Turkish (**c**) populations. Each inverted triangle corresponds to a single breakpoint in one given intron.

breakpoints in intron 50, with a moderate peak of breakpoints in intron 44 (Fig. 2c). Therefore, the presence of a hot spot within intron 50 seems a peculiar characteristic in the samples from the Balkan region.

The size of intron 44 was estimated to be at least 200 kb in length, whereas the segment containing introns 45–51 encompasses, at most, 270 kb of DNA [Nobile et al., 1995]. According to data from the literature [Danieli et al., 1993], in this subregion the incidence of deletion breakpoints is 2.6 times higher than in intron 44 (the major deletional hot spot in the dystrophin gene), while its size is only 30% larger.

According to the data from the present report, 13.2% of deletion breakpoints fall in intron 44, and 54.7% in the segment including introns 45–51. The distribution of breakpoints by intron within this subregion points at the probable existence of an important deletional hot spot in intron 50, since 29.3% of the breakpoints mapped in this subregion fall there.

Due to the absence of selection of intronic sequences and to genetic drift, some extragenic DNA segments may have accumulated relevant differences in time, and may have evolved into deletion-prone segments [Danieli et al., 1993]. Therefore, their distribution along the gene would be expected to be different in different populations. The present data seem to confirm this view, but only a more detailed genomic analysis of the distribution of breakpoints along the dystrophin gene will clarify the issue.

ACKNOWLEDGMENTS

We thank Dr. U. Lenk for providing us with PCR primers, and Dr. F.A. Saad for helpful technical suggestions. Financial support from the Italian Ministry of University and Scientific Research and the Bulgarian Ministry of Education and Sciences is gratefully acknowledged. A.T. was the recipient of a fellowship from TELETHON-Italy.

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